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Properties of Hepatitis B Virus Genome Recovered From Vietnamese Patients With Fulminant Hepatitis in Comparison With Those of Acute Hepatitis

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Among the many mutations found in the hepatitis B virus (HBV) genome, some have been associated with fulminant hepatitis, as exemplified by precore-defective mutations. The aim of this study was to determine whether such mutations also are found in Vietnamese cases of fulminant hepatitis B. The full-genome nucleotide sequence of HBV in three patients with fulminant hepatitis (F-2, F-3, and F-6) and one with acute hepatitis (A-3), who were admitted to Cho Ray Hospital, Ho Chi Minh City, Vietnam was ascertained. Additionally, two patients with fulminant hepatitis (F-1 and F-7) and three with acute hepatitis (A-1, A-2, and A-5) were examined only for the precore/core region of HBV. Remarkably, the nonsense mutation at precore codon 28 (Trp82Stop) was found in four of the five patients with fulminant hepatitis, while all the acute hepatitis patients harbored wild type (one had a mixture of wild and mutant types). The missense mutations within the core region, Ile97Leu and Pro130Ile/Thr/Ser, were also remarkable in fulminant hepatitis. Only F-2 was free from these precore/core mutations, but F-2 was unique in that it possessed a chimeric genotype: it could be classified into genotype C as a whole, but its X region was of genotype B, like the other four fulminant hepatitis isolates (F-1, F-3, F-6, and F-7). The codon 41 of the X protein was Pro in all three fulminant hepatitis cases examined for this region, while it was Ser in the wild-type isolates of genotype B. Of note as negative data, the mutations C1653T and T1753M of the enhancer II (Enh II) and A1762T and G1764A of the precore/core promoter regions, once reported to be relevant to severe or fulminant hepatitis, were not found in the present cases. The results with the Vietnamese cases of fulminant hepatitis corroborated results of previous studies with respect to the mutations Trp28Stop of precore and Ile97Leu and Pro130Ile/Thr/Ser of core, but not for the mutations within Enh II and precore/core promoter region. Whether the Ser41Pro mutation in the X region of genotype B HBV is Vietnam-specific or disease-specific deserves further investigation. ***J. Med. Virol. 61:23–28, 2000.***

KEY WORDS: hepatitis B virus (HBV); fulminant hepatitis; Vietnam

INTRODUCTION

Hepatitis B virus (HBV) induces acute liver disease, ranging from subclinical to fulminant hepatitis, as well as chronic disease in persistently infected hosts, which may lead to hepatocellular carcinoma in the worst scenario. Accumulating evidence indicates that certain mutations in the HBV genome may influence or reflect

The nucleotide sequence data of the four full-genome isolates hepatitis B virus reported in this article will appear in the DDBJ/EMBL/GenBank databases under accession numbers AB031260 through AB031268.

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TABLE I. Demographic, Clinical, and Virologic Characteristics of the Four Patients With Acute Hepatitis and Five With Fulminant Hepatitis

	Acute hepatitis				Fulminant hepatitis				
Characteristics	A-1	A-2	A-3	A-5	F-1	F-2	F-3	F-6	F-7
Age (yr) and sex	21-f	27-m	33-m	27-m	29-m	25-m	29-m	26-m	35-f
Clinical data[a]									
Total bilirubin (mg/dL)	3.4	3.6	27.0	15.1	16.8	15.9	25.2	36.0	15.4
ALT (IU/L)	521	423	1,263	1,410	136	1,267	1,117	1,485	638
Prothrombin time (%)	90	85	57	67	21	36	10	1	40
HBeAg/Ab	–/+	+/–	+/–	+/–	–/+	–/+	–/+	+/–	–/–
HBsAg subtype	n.d.	n.d.	adr	n.d.	n.d.	adr	ayw	ayw	n.d.
HBV genome length (nucleotides)	n.d.	n.d.	3,215	n.d.	n.d.	3,215	3,155[b]	3,215	n.d.
Genotype	B	C	C	C	B	C(B)[c]	B	B	B
Enh II and pC/C promoter									
C1653T [Ferrari et al., 1991]	–	–	–	n.d.	–	–	–	–	n.d.
T1753M [Gerkin et al., 1991]	–	–	–	–	–	–	–	–	–
A1762T [Gerkin et al., 1991]	–	–	–	–	–	–	–	–	–
G1764A [Gerkin et al., 1991]	–	–	–	–	–	–	–	–	–
Precore									
Trp28Stop [Carman et al., 1989, 1995; Ehata et al., 1992]	±[d]	–	–	–	+	–	+	+	+
Core									
Ile97Leu [Kakumu et al., 1998]	–	–	–	–	–	–	–	+	+
Pro130Non-Pro [Kakumu et al., 1998]	–	–	–	+	–	–	+	+	+
Ser183Pro [Kaneko et al., 1995]	–	–	–	–	–	–	–	–	–
PreS									
Met1Non-Met [Kosaka et al., 1991]	–	–	–	–	–	–	+	–	+

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; Ab, antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; Enh II, enhancer II; n.d., not determined.
[a]Data on admission.
[b]With a 60-nucleotide deletion in the preS region.
[c]Its X region suggests genotype B, while other regions suggest C.
[d]Mixture of wild and mutant types.

the natural course and/or clinical manifestations of infection. "Precore-defective" mutants, with a point mutation [Brunetto et al., 1989; Carman et al., 1989] or frame-shift mutations [Okamoto et al., 1990] that abolish the synthesis of hepatitis B e antigen (HBeAg), were first noted to be relevant to fulminant hepatitis [Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991]. Later studies suggested that still other mutations also may be associated with fulminant hepatitis or acute exacerbation of chronic hepatitis: C1653T [Kaneko et al., 1995] and T1753C/A/G [Sato et al., 1995] in the enhancer II (Enh II); A1762T and G1764A in the precore/core promoter region [Sato et al., 1995]; Trp28Stop of precore [Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991]; Ile97Leu, Pro130Non-Pro [Okumura et al., 1996], and Ser183Pro of core [Aye et al., 1994]; and defects in preS2 [Pollicino et al., 1997]. It remains uncertain, however, whether these mutations are causatively associated with fulminant hepatitis or only passively reflect the results of immune selection [Baumert and Liang, 1996].

Previous reports on these fulminant hepatitis–associated mutants mostly have originated from Japan and Western countries. Therefore, we felt it worthwhile to examine Vietnamese HBV isolates. In addition, since the reported data have been based mostly on fragmentary sequences of the HBV genome, it was considered important to sequence our isolates for the entire genome. The complete sequences of HBV from three patients with fulminant hepatitis and one patient with acute hepatitis, together with the precore/core region sequences from several other patients, are described.

MATERIALS AND METHODS

Patients

Five patients with fulminant hepatitis B (F-1, F-2, F-3, F-6, and F-7) and four with acute hepatitis B (A-1, A-2, A-3, and A-5), admitted to Cho Ray Hospital in Ho Chi Minh City, Vietnam, during the period from 1994 to 1996, were enrolled in this study (demographic and clinical data are listed in Table I). All of the fulminant hepatitis patients experienced hepatic encephalopathy (coma grades 2–4) within 8 weeks of the onset of illness, and a remarkable prolongation of prothrombin

time (<40%). Three of them (F-1, F-3, and F-6) died. Although one acute hepatitis patient (A-3) showed a significant degree of bilirubinemia (27.0 mg/dL) and prothrombin time prolongation (57%), almost comparable to those in fulminant hepatitis, encephalopathy did not develop, and hepatitis resolved rapidly, as in the other cases of acute hepatitis. None of the patients had a history of liver disease. Serum samples taken from these patients on admission were stored at –20°C or below for later determination or viral markers and genomic sequences.

Full-genome Sequencing

Nucleic acids were extracted from 50 μL of serum samples using SmiTest EX R&D (Sumitomo Metal Industries, Ltd., Kashima, Ibaragi, Japan). To obtain full-length HBV DNA sequences from the nucleic acids of patients F-2, F-3, F-6, and A-3, a long-distance nested polymerase chain reaction was performed using a procedure described previously [Takahashi et al., 1998]. Briefly, two amplicons were obtained by nested PCR with use of the *Taq* Plus Long PCR System (Stratagene, LaJolla, CA). Amplicon A, of about 2.6 kb in length and corresponding to nucleotide (nt) positions 455→3094 of a standard genotype C HBV isolate, HPBADRA (database accession number M12906), was externally primed by S1-1 (5′-TCGTGTTACAGGCGGGGTTT-3′, nt 192-211, sense) and T734 (5′-CTTCCTGACTGSCGATTGG-3′, nt 3137-3155, antisense), and internally primed by S2-1 (5′-CAAGGTATGTTGCCCGTTTG-3′, nt 455-474, sense) and T733 (5′CCTGAGCCTGAGGGCTCCAC-3′, nt 3075-3094, antisense). Amplicon B, of about 2.3 kb in length (nt 1606→687) was externally primed by T715 (5′-CTGTGCCTTCTCATCTGCCG-3′, nt 1553-1572, sense) and S1-2 (5′-CGAACCACTGAACAAATGGC-3′, nt 685-704, antisense) and ep1-1 (5′-GCATGGAGACCACCGTGAAC-3′, nt 1606-1625, sense) and S2-2 (5′-GGCACTAGTAAACTGAGCCA-3′, nt 668-687, antisense). Genetic mapping of HBV with the location of the amplicons A and B is shown in Fig. 1.

These amplicons were purified by Microcon 100 (Amicon Corp., Beverly, MA) and subjected to direct sequencing with use of the Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Inc., Foster City, CA) and the 373A DNA sequencer (Applied Biosystems, Foster City, CA). The nucleotide sequences of the two amplicons were merged at the overlapping regions to complete the circular genome of HBV.

Precore/core Region Sequencing

The 2.3-kb amplicon B also was obtained from patients F-1, F-7, A-1, A-2, and A-5 by the methods described but was sequenced only for a region that covers the open reading frames of precore and core, using primers described elsewhere [Takahashi et al., 1998].

Analyses of Sequences

Genotyping of HBV DNA into A through F [Okamoto et al., 1988; Norder et al., 1994] was done by phylogenetic comparison of obtained sequences against those with known genotypes: HBVADW2 (X02763, genotype A), HPBADW1 (D00329, B), HPBADRA (M12906, C), XXHEPAV (X02496, D), HHVBE4 (X75664, E), and HHVBF (X75663, F). Phylogenetic trees were constructed by the neighbor-joining method and the unweighted pair–grouping method with arithmetic means using GENETYX-MAC version 8.0 (Software Development, Shibuya, Tokyo).

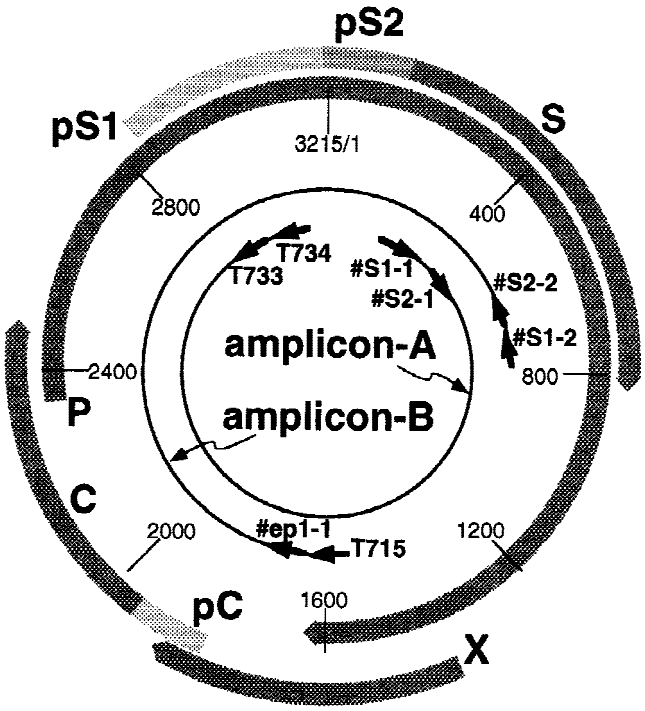

Fig. 1. Genetic map of hepatitis B virus with the location of polymerase chain reaction-amplicons A and B.

RESULTS

Of the four full-genome isolates, three (F-2, F-6, and A-3) had a length of 3215 nt. F-3 was 3155 nt in length, 60 nt shorter than the others due to a deletion within the presS region. This deletion abolished the start codon of the preS2 protein. With the precore/core region sequences from F-1, F-7, A-1, A-2, and A-5 taken together, the properties of the HBV genome were compared between fulminant hepatitis and acute hepatitis (Table I). Genotype C was found in three of the four acute hepatitis isolates but in only one (F-2) of the five fulminant hepatitis isolates; the others were genotype B. Even F-2 could be classified under genotype B when the X regions were compared, as detailed later herein.

Among the mutations so far reported to be relevant to fulminant hepatitis or other severe forms of hepatitis, a precore-defective mutation was most prominent in the present cases of fulminant hepatitis: Trp28Stop of precore was found in all the fulminant cases other than F-2 but in only one acute case (A-1). A-1 harbored the Trp28Stop mutant in a mixture with a wild-type sequence. The Ile97Leu mutation of core and the abo-

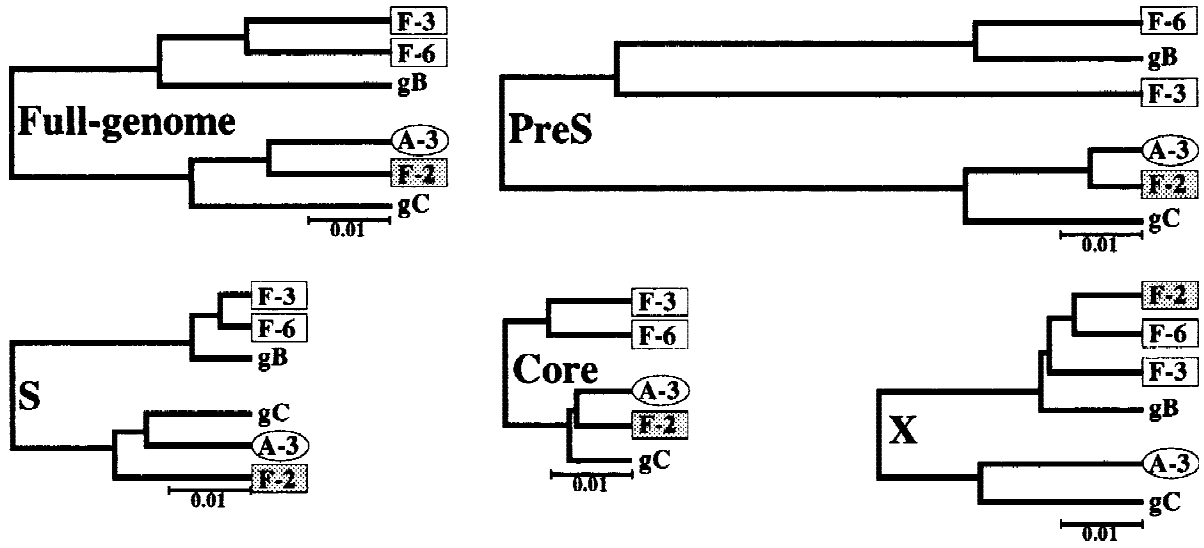

Fig. 2. Phylogenetic trees (unweighted pair–grouping method with arithmetic means) for the four full-genome Vietnamese hepatitis B virus isolates constructed on different regions of the genome. F-2 (shaded) and A-3 (circled) were grouped together in every tree other than that based on the X region. gB: HPBADW1 (D00329, genotype B); gC: HPBADRA (M12906, genotype C).

lition of the preS2 start codon were found in two fulminant cases but none of the acute cases. Pro130Non-Pro of core was found in three fulminant cases versus one acute case. Ser183Pro was found neither in fulminant nor acute hepatitis. No remarkable mutations were found in the Enh II and precore/core promoter region in our present isolates, though an association with fulminant hepatitis has been suggested for such mutations as C1653T, T1753M, A1762T, and G1764A.

The F-2 isolate, lacking mutations that have so far been reported to be linked to fulminant hepatitis, possessed an unexpected and unique property. As shown in Fig. 2, F-2 was classified under the same branch with A-3 and a standard sequence of genotype C when phylogenetic trees were generated based on the full-genome, preS, S, and core regions, but it was grouped with F-3 and F-6 under the branch of genotype B, apart from A-3 for the first time, when the X region was used for comparison. That is, F-2 had a chimeric genome, as if a genotype C HBV DNA had undergone recombination with a genotype B HBV only at the X region.

Genotype B isolates available from databases were compared with the three Vietnamese isolates from fulminant hepatitis for the predicted amino acid sequence of the X protein (Fig. 3). Ser41Pro was found in all the Vietnamese isolates but in none of the others (Fig. 3a). A phylogenetic tree based on the amino acid sequence of the X protein suggested that clustering of isolates by comparing the X region reflects a geographical difference rather than a clinical difference (Fig. 3b).

DISCUSSION

The clinical impact of HBV infection still outweighs that of HCV in Vietnam, according to a previous study [Kakumu et al., 1998]. In that study, 46 patients with hepatitis B surface antigen and acute liver disease with overt jaundice were identified; five with fulminant and four with acute hepatitis were selected for the present study. The Vietnamese HBV isolates recovered from fulminant hepatitis patients examined in this study had some, but not all, characteristics that have been reported to be associated with a fulminant or severe form of hepatitis. Namely, Trp28Stop (precore), Ile97Leu (core), Pro130Non-Pro (core), and Met1Non-Met (preS2) were the mutations that were positive in our patients, and C1653T, T1753M, A1762T, G1764A (Enh II and pC/C promoter), and Ser183Pro (core) were those that were not identified at all (Table I).

With respect to Trp28Stop (precore), all but one (F-2) fulminant hepatitis isolate had it. One (A-1) of the four acute hepatitis isolates also had it but only concurrently with a wild-type sequence. Of note, F-6 was HBeAg-positive despite the precore-defective mutation (Table I). One possible explanation for this apparent conflict is that a wild-type sequence might also be present in F-6 or that the as yet unassembled core polypeptide might have been shed into the circulation and detected by the HBeAg assay.

The missense mutations in the core region, Ile97Leu and Pro130Non-Pro, were found in two and three of the five fulminant cases respectively, but in only one of the four acute hepatitis cases. These mutations have been reported to evolve during acute exacerbations of chronic hepatitis [Okumura et al., 1996] and to exist very often in patients with hepatocellular carcinoma [Takahashi et al., 1998]. Missense mutations at particular positions of the core-coding region, including Ile97Leu and Pro130Non-Pro, may be responsible for the development of severe liver disease, since the core protein serves as an important target of immune response by lymphocytes [Ferrari et al., 1991; Wakita et al., 1991; Ehata et al., 1992; Carman et al., 1995].

The preS2 defect was found in two cases of fulminant hepatitis: the initiation codon for preS2 was missing by

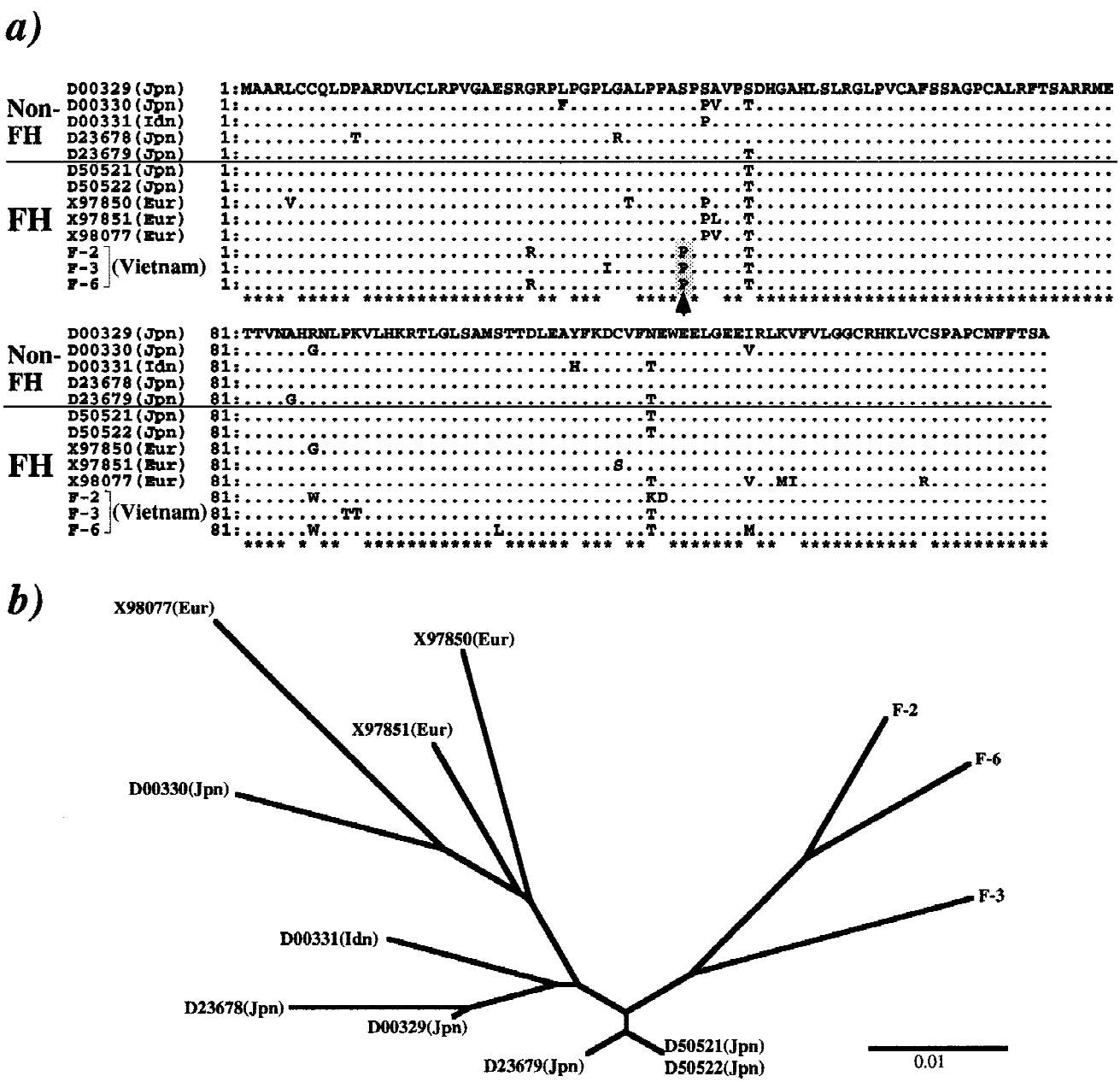

Fig. 3. Comparison of the amino acid sequence of the X protein encoded by genotype B HBV isolates. **a:** Alignment of the amino acids. FH, fulminant hepatitis; Jpn, Japan; Idn, Indonesia; Eur, Europe. Proline at codon 41 is highlighted by shade and arrow. **b:** Phylogenetic tree (by neighbor-joining method).

a deletion in one (F-3) and by a point mutation in another (F-7). PreS-defective mutants have been reported [Gerken et al., 1991; Takayanagi et al., 1993]: in particular, Pollicino et al. [1997] suggested an important role for the preS2-defective mutants in the pathogenesis of fulminant hepatic failure.

Among the five isolates from fulminant hepatitis, only one (F-2) was completely free from the mutations mentioned. In fact, the F-2 isolate could be regarded as a wild type at first glance. F-2 possessed a unique feature in its genetic organization. It was a chimera between genotypes B and C or, more precisely, a recombination mutant of genotype C with its X region replaced by that of genotype B (Fig. 2). This prompted us to speculate that the X region exerts a key role in the pathogenesis of fulminant hepatitis and to compare amino acid sequences of the X protein between isolates from patients with fulminant hepatitis and those from asymptomatic carriers or from patients with liver diseases other than fulminant hepatitis. Results indicated that the clusters in the phylogenetic tree based on the X protein reflect geographical difference rather than differences in disease expression (Fig. 3). However, the Ser41Pro mutation, shared in common by all three Vietnamese fulminant hepatitis isolates examined for this region, remains intriguing. Further studies are

warranted to determine whether the proline at codon 41 of the X gene is very common in Vietnamese genotype B HBV isolates regardless of disease severity or specific to those from fulminant hepatitis.

In conclusion, the present results add to the long list of the precore-defective mutants possibly playing an important role in the pathogenesis of fulminant hepatitis. Whether it is a product of chance or of necessity that all fulminant hepatitis isolates examined here shared genotype B at the X region is yet to be determined.

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REFERENCES

Aye TT, Uchida T, Becker SO, Hirashima M, Shikata T, Komine F, Moriyama M, Arakawa Y, Mima S, Mizokami M, Lau JYN. 1994. Variations of the hepatitis B virus precore/core gene sequences in acute and fulminant hepatitis B. Dig Dis Sci 39:1281–1287.

Baumert TF, Liang TJ. 1996. Precore mutants revisited. Hepatology 23:184–186.

Brunetto MR, Stemler M, Schodel F, Will H, Ottobrelli A, Rizzetto M, Verme G, Bonino F. 1989. Identification of HBV variants which cannot produce precore derived HBeAg and may be responsible for severe hepatitis. Ital J Gastroenterol 21:151–154.

Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. 1989. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet 2:588–591.

Carman WF, Thursz M, Hadziyannis S, McIntire G, Colman K, Gioustoz A, Fattovich G, Alberti A, Thomas HC. 1995. Hepatitis B e antigen negative chronic active hepatitis: hepatitis B virus core mutations occur predominantly in known antigenic determinants. J Viral Hepatitis 2:77–84.

Ehata T, Omata M, Yokosuka O, Hosoda K, Ohto M. 1992. Variations in codon 84-101 in the core nucleotide sequence correlate with hepatocellular injury in chronic hepatitis B virus infection. J Clin Invest 89:332–338.

Ferrari C, Bertoletti A, Penna A, Cavalli A, Valli A, Missale G, Pilli M, Fowler P, Giuberti T, Chisari FV, Fiaccadori F. 1991. Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. J Clin Invest 88:1793–1801.

Gerken G, Kremsdorf D, Capel F, Petit MA, Dauguet C, Manns MP, Meyer zum Buschenfelde KH, Brechot C. 1991. Hepatitis B defective virus with rearrangement in the pre-S gene during chronic HBV infection. Virology 183:555–565.

Kakumu S, Sato K, Morishita T, Anh TK, Binh NH, Dien BV, Chinh DH, Phuc NH, Tinh NV, Trinh LT, Yamamoto N, Nakao H, Isomura S. 1998. Prevalence of hepatitis B, hepatitis C, and GB virus/hepatitis G virus infections in liver disease patients and inhabitants in Ho Vhi Minh, Vietnam. J Med Virol 54:243–248.

Kaneko M, Uchida T, Moriyama M, Arakawa Y, Shikata T, Gotoh K, Mima S. 1995. Probable implication of mutations of the X open reading frame in the onset of fulminant hepatitis B. J Med Virol 47:204–208.

Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. 1991. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 100:1087–1094.

Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath EA. 1991. Hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N Engl J Med 324:1705–1709.

Norder H, Couroucé A-M, Magnius LO. 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 198:489–503.

Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. 1988. Typing hepatitis B virus by homology in nucleotide sequencing: comparison of surface antigen subtypes. J Gen Virol 69:2575–2583.

Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. 1990. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 64:1298–1303.

Okumura A, Takayanagi M, Aiyama T, Iwata K, Wakita T, Ishikawa T, Yoshioka K, Kakumu S. 1996. Serial analysis of hepatitis B virus core nucleotide sequence of patients with acute exacerbation during chronic infection. J Med Virol 49:103–109.

Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. 1991. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 324:1699–1704.

Pollicino T, Zanetti AR, Cacciola I, Pett MA, Smedile A, Campo S, Sagliocca L, Pasquali M, Tanzi E, Longo G, Raimondo G. 1997. Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. Hepatology 26:495–499.

Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. 1995. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 122:241–248.

Takahashi K, Akahane Y, Hino K, Ohta Y, Mishiro S. 1998. Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. Arch Virol 143:2313–2326.

Takayanagi M, Kakumu S, Ishikawa T, Higashi Y, Yoshioka K, Wakita T. 1993. Comparison of envelope and precore/core variants of hepatitis B virus (HBV) during chronic HBV infection Virology 196:138–145.

Wakita T, Kakumu S, Shibata M, Yoshioka K, Ito Y, Shinagawa T, Ishikawa T, Takayanagi M, Morishima T. 1991. Detection of pre-C and ore mutants of hepatitis B virus in chronic hepatitis B virus carriers. J Clin Invest 88:1793–1801.